Journal of
Plant Sciences

ISSN 1816-4951

Academic
Journals Inc.

www.academicjournals.com

Journal of Plant Sciences 6 (4): 182-189, 2011
ISSN 1816-4951 / DOI: 10.3923/jps.2011.182.189

The Relationship Between Some Mycotoxins Excretion and Bean Seed Discoloration

[1]Muneera D.F. Alkahtani, [2]M.M. Mazen, [3]M.A. El-Naggar and [2]M.K. Arfa
[1]Faculty of Sciences, Prince Nora University, KSA
[2]Plant Pathology Research Institute, ARC, Egypt
[3]Research Center Lab, GSFMO, KSA P.O. Box 3402 Riyadh 11471, KSA

Corresponding Author: M.A. El-Naggar, Research Center Lab, GSFMO, KSA P.O. Box 3402 Riyadh 11471, KSA Tel: +966564890050, +966121033331/1010

ABSTRACT

Mycotoxins is a serious problem threatening human and animal health and detection is the most important steps to get rid of them. The impact of these mycotoxins on seed physiological characteristics as discoloration is important things that are related with seed quality as well as marketing. The fungal isolation was conducted from three cultivars of bean seeds ($Giza_3$, $Giza_4$ and $Giza_6$) after dividing into groups depending on their color. Sixteen fungal species were associated with discolored bean seed samples. The Aflatoxin (AF), Alternariol (AOH) and Zearalenone (ZON) were determined using immunoaffinity columns and GC-MS. Aflatoxin was existed in all colored seeds while, the dark brown colored seeds contaminated with AF, AOH and ZON. The highest concentration was found in dark brown colored seeds followed by golden brown. However, Zearalenone (ZON) was detected only in the dark brown in high concentration (45 μg kg^{-1} seeds). AOH toxin excreted by both isolates of *A. alternata* ranged from 1.0 to 3.0 μg g^{-1}. The highest level of AOH toxin has been detected after 14 days of incubation and then decreased. Whereas, the normal seeds contained the lowest concentration of Aflatoxin. The amount of Aflatoxin produced by *A. parasiticus* increased positively with increasing incubation time. The amount reached 200 (μg kg^{-1}) after 35 days incubation. Nevertheless, this amount was decreased to 125 (μg kg^{-1}) after some period when bean seeds simultaneously inoculated with *A. alternata* and *A. parasiticus*. In conclusion mycotoxins affect on the seed physiology, there is a direct relationship between the rate of mycotoxins (AF, AOH and ZON) contamination and the degree of discoloration. The presence of more than fungus together may result in lack of mycotoxins content.

Key words: *Phaseolus vulgaris*, alternariol, zearalenone, aflatoxins, seed discoloration

INTRODUCTION

Bean (*Phaseolus vulgaris* L.) seeds consider one of food legume crops had a high nutritional value specially starch, protein and dietary fiber and is an excellent source of iron, potassium, selenium, molybdenum, thiamine, vitamin B6 and folic acid. 18.3 million tones of dry common beans and 6.6 million tones of green beans were grown worldwide in 2007 because of their high protein content and relatively low cost, they are consumed in substantial amounts. The preparation of dried beans for eating usually is similar to green beans which were prepared and cooked in water. Considering their method of preparation and the amount consumed, the question arose concerning the presence and relative prevalence of toxicogenic molds

in or on these foodstuffs. The literature indicates that at present little is known about the mold flora of dried beans and its potential to produce mycotoxins. The commercial production of beans is well distributed with countries in Asia, Africa, Europe, Oceania, South and North America all among the top bean growers (FAO, 2009).

Numerous fungi has associated with bean seeds and pods *i.e., Fusarium solani*, *F. oxysporum*, *F. equiseti*, *Pythium* sp., *Aspergillus* sp. and *Alternaria tenuis* (El-Mougy, 2001; Patkowska, 2006). Seed-borne fungi play an important role in deterioration of seed quality, which lead to high economic loss in crop yield (Youssef *et al*., 2008). These fungi capable of causing seed discoloration and reduce viability and germination of the seeds (Bruchera and Comacho, 2000; Icishahayo *et al*., 2009). Also, the fungi can greatly affect quality of the harvest bean as well as producing mycotoxins. These toxic fungi may be also associated with harmful in biochemical changes in fruits and seeds such as protein, lipid, fiber and vitamins (Reddy *et al*., 2010; Alkahtani *et al*., 2011). The toxic influence of *Alternaria alternata* and *Aspergillus parasiticus* as was studied by Dalcero *et al*. (1989) and Youssef (2009). They found that *A. alternata* would not compete with *A. parasiticus* in colonization of the seed but would either degrade the aflatoxin by *A. parasiticus* or compete for aflatoxin biosynthesis precursors. In the latter situation the fungi also produced the mycotoxins Zearalenone (ZON) and Alternariol (AOH) (Bottalico *et al*., 1989). Furthermore, *F. oxysporum*, *Aspergillus flavus*, *A. parasiticus* and *A. alternate* associated with seeds were recorded to produce some mycotoxins such as zearalenone, aflatoxin and alternariol. External discoloration reduces the aesthetic of the bean. Moreover, infected seeds with specific pathogens which produce toxin may affect the health of human and/or animals consumers (Bryden, 2007; Alwakeel and Nasser, 2011).

The relation between color seeds and mycotoxin content in this investigation was early reported by Arafa *et al*. (1998). They found that Alternariol (AOH), Alternariol Monomethylether (AME) and Zearalenone (ZON), toxins in dark brown discolored seeds of soybean. In industrialized countries, stringent sorting and clean up procedures are used to reduce aflatoxins to low levels in foods with a perceived risk. The seed discoloration was investigated later by Reddy *et al*. (2005) and Mangala *et al*. (2006). For peanuts, where fungal growth is usually accompanied by discoloration of the kernel, this includes the use of sophisticated color sorting equipment (Pitt, 2000). Since seed discoloration is strongly associated with AFB1 contamination rates, color sorting blanched peanuts is even more (up to 91%) effective than sorting unbalanced peanuts by color (Dorner, 2008).

The objectives of this study were to investigate the relation between bean seed discoloration and fungi associated with these seeds, influence of *A. alternate* on aflatoxin production by *A. parasiticus* and to determine the mycotoxin content in the deformed bean seeds.

MATERIALS AND METHODS

Sources of samples: Seed samples of three bean cultivars ($Giza_3$, $Giza_6$ and $Giza_4$) were collected from seed lots at different locations in Assuit governorate, Egypt in 2009. The seeds were divided to three groups based on their colors slight brown, deep brown and normal color. Each group contained 400 seeds according to the International Role for seed testing association (ISTA, 1976).

Isolation and identification of moulds: The seed samples were surface sterilized with 2% sodium hypochlorite solution for one min., dried between filter papers, then placed onto autoclaved Petri dishes contained Potato Dextrose Agar (PDA) medium. The plates were incubated at 25±2°C for seven days. The developed fungal colonies were isolated, purified using hyphal tip transfers

method mentioned by Brown (1924) or by single spore technique described by Ezekiel (1930). Identification of the isolated fungi was carried out in Plant Pathology Research Institute, Agricultural Research Center, Giza, according to Barnett and Hunter (1972).

Tow isolates of both *Alternaria alternate* and *Aspergillus parasiticus* were used in this investigation. Both isolates were previously isolated from bean seed color and tested to determine their ability to produce (AOH) and aflatoxin. Twenty-five gram sterilized rice was placed in 250 mL. Erlenmeyer flask was inoculated with 7 mm disks of each *A. alternate* or *A. parasiticus* cultures and incubated at 28±2°C for seven days under relative humidity of 90±2%. At the end of the incubation period, the growth of each fungus was screened for.

Germination: Four hundred seeds from each of the three tested groups were placed on wet filter paper in Petri dishes. The plates were incubated at room temperature. Percentage of seed germination was determined according to the method described by Bechtel *et al.* (1989). Seedling damage (root and/or hypocotyls) was recorded 10 days after incubation. Discolored embryos were estimated by using the description of Christensen (1967). The pericarps covering the embryo was removed and examined with and without the aid of a stereoscopic dissecting microscope.

Mycotoxin analysis: The tested mycotoxins (AF, AOH and ZON) were determined using immunoaffinity columns and a standard (R-Biopharm). Mycotoxins were extracted from samples with appropriate solvents. After sample clean-up, mycotoxins were determined by gas chromatography with mass spectrometry (GC-MS), using system HP 6890 Series (Hewlett Packard, CA, USA) with mass selective detector 5975B inert XL (Agilent Technologies, CA, USA). The column was HP-5MS, 30 m, 0.25 mm I.D., 0.25 μm (Agilent Technologies) and helium with a flow rate of 1 mL min^{-1} was used as the carrier gas according to Tanaka *et al.* (2000), Melchert and Pabel (2004) and Schothorst *et al.* (2005).

Statistical analysis: The statistical analysis was performed using the Analysis of Variance (ANOVA) with statistical program (MSTAT-C) package The least significant difference procedure (LSD) was used at 0.05 level of probability.

RESULTS

The tested bean seeds divided according to the degree of color to the 3 groups, the first one had a normal color, the second was golden brown while the third group is the most shop or dark brown. Sixteen fungal species frequently associated with discolored bean seed. The samples were screened and listed in Table 1. *Alternaria alternata* and *Aspergillus parasiticus* were the most frequently isolated fungi followed by *Aspergillus flavus, Fusarium oxysporum, F. solani* and *Curvularia lumata*. Nevertheless, *Penicillium oxalicum* and *Mucar racemosus* were the lowest isolated fungi. *Pythium* sp. and *Rhizoctonia solani* were isolated only from deep brown colored seeds. Whereas, *Cladosporium cladosporioides* was found only in the light brown colored seeds. The most frequent fungi in normal bean group was *A. tenuissima* (3.0%) while, *Alternaria alternata* was predominate (12.5 and 17.1%, respectively) in both golden brown and dark bean and followed by *Aspergillus parasiticus* 10.3 and 13%, respectively.

The percentage of germination was 96.4, 70.5 and 42.2% in normal, golden brown and dark brown groups, respectively. The highest percentage of seedling damage was in dark brown followed by golden brown and normal groups as 36.1, 22.2 and 2.0%, respectively. The discoloration

Table 1: Frequency of the fungi isolated from infected discolored bean seeds

	% Frequency of different type of discolored bean seeds		
Isolated fungi	Normal	Golden brown	Dark brown
Alternaria alternata	2.0	12.5	17.1
Aspergillus tenuissima	3.0	2.9	3.5
Aspergillus flavus	2.0	8.0	11.5
Aspergillus niger	1.0	2.0	2.5
Aspergillus parasiticus	2.0	10.3	13.0
Cladosporium cladosporioides	0.0	1.8	0.0
Cladosporium sphaerospernmum	0.0	1.0	2.1
Curvularia lumata	0.0	3.3	3.3
Fusarium solani	2.0	4.8	6.1
Fusarium oxysporum	0.0	6.8	9.6
Mucar racemosus	0.0	1.0	1.0
Penicillium oxalicum	0.0	1.0	1.0
Penicillium chrysgenum	0.0	1.5	1.9
Pythium sp.	0.0	0.0	1.3
Rhizoctonia solani	0.0	0.0	1.3
Rhizopus stolonifer	1.0	1.0	2.1
Other fungi and unknown	87.0	42.1	22.7

Table 2: Germination and dark embryos of colored bean seeds

			Dark embryos[b]	
Seed discoloration	% Germination[a]	% Seedling damage	Tip	All
Normal	96.4	2.0	1.0	0.0
Golden brown	70.5	22.2	10.0	5.0
Dark brown	42.2	36.1	21.0	16.0
LSD at 5%	12.3	9.4	8.2	5.9

[a]Mean of four replicates of 100 seed each after incubation for 7 days at 25°C. [b]Mean of 100 seed

Table 3: Mycotoxins detected in discolored bean seeds

	Concentrations of mycotoxins (μg kg^{-1})		
Discolored bean seeds types	Aflatoxins	Alternariol	Zearalenone
Normal	10.0	0.0	0.0
Light brown	35.0	50.0	0.0
Dark brown	60.0	85.0	45.0
LSD at 5%	4.3	4.8	5.1

extended to embryos axis in case of dark brown and golden brown as 16.0 and 5.0% while the tip discoloration was 21 and 10%, respectively (Table 2).

Mycotoxins analysis of colored bean seed presented in Table 3 show that the highest concentration of Aflatoxin and Alternariol (AOH) and Zearalenone were existed in dark brown seeds (60, 85 and 45 μg kg^{-1}, respectively). In case of golden brown the concentration of both Aflatoxins and Alternariol was decreased to 35.0 and 50.0 μg kg^{-1}, while the concentration of Zearalenone was disappear. In normal bean seeds, the aflatoxins was determined only as

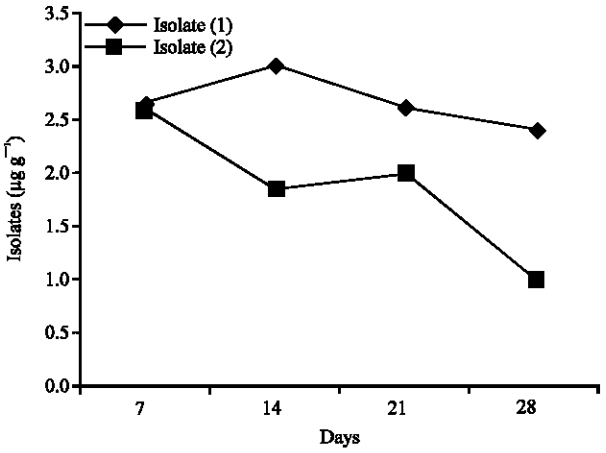

Fig. 1: Alternariol (AOH) production (µg g^{-1}) by two different isolates (1, 2) of *Alternaria alternata* isolated from bean seeds during different periods

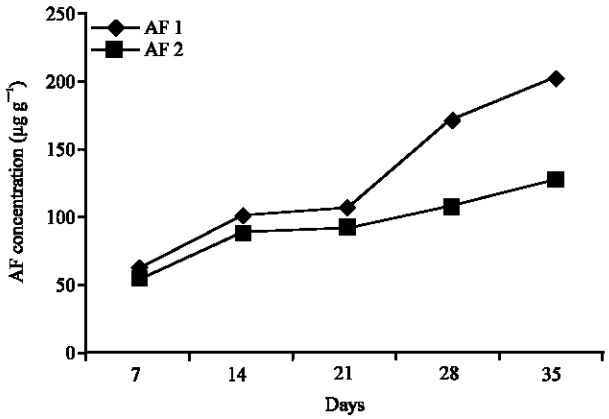

Fig. 2: Aflatoxins (AF1) concentration (µg g^{-1}) produced in inoculated been seeds with pure culture of *Aspergillus parasiticus* only. Aflatoxins (AF2) concentration (µg g^{-1}) produced in inoculated been seeds with cultures of *Aspergillus parasiticus* and *Alternaria alternata* together

10 µg kg^{-1}. Figure 1 shows the amount of Alternariol (AOH) toxin produced by both isolates of *A. alternate*. The level of (AOH) ranged from 1.0 to 3.0 g. The highest level of Alternariol toxin was detected that 14 days after incubation and decreased after words in both isolates. The isolate No. 1 of Alternaria alternata was more producers for Alternariol toxins than other isolate No. 2 over 28 days.

Effect of simultaneous inoculation of bean seed with *A. parasiticus* and *A. alternata* aflatoxin is illustrated in Fig. 2. Amount of aflatoxin produced by *A. parasiticus* increased positively with increasing incubation time. The amount reached 200 (µg kg^{-1}) after 35 days incubation. Nevertheless, this amount was decreased to 125 (µg kg^{-1}) after some period when bean seeds simultaneously inoculated with *A. alternata* and *A. parasiticus* together.

DISCUSSION

Abnormal seedlings were generally covered with fungal growth. Seedlings, which escaped from seed decay, were frequently developed necrotic lesions on the cotyledons and occasionally the terminal bud or roots become infected resulting in eventual death of the seedlings. Adequate knowledge of the quality of the seed stock is essential in order to obtain crops free from seed-borne diseases. It is well documented that seed-born fungi (internally and/or externally) responsible for poor germination, seed rot, seedling damping-off, plant stand and subsequently yield loss, as well as reducing the grade (quality) of the seeds in the market. These results were consistent with Hemannavar (2008), Abdulsalaam and Shenge (2011) and Govindappa *et al.* (2011).

Infected seeds can classified according to the appearance of shape, size, shrivel and deformation of the testa. In the present investigation, the count of fungi recorded from bean seeds was sixteen different fungi. These considerable fungal numbers probably due to the high moisture content and nutritional value of bean seeds which conductive to be colonized by fungi and this reasoning may be compatible with the sentiments of Zhang (1992) and Philippoussis (2009). There is no particular color for each fungus was isolated and most colorful seeds tended to blackish or brownish with all the fungi isolated. In isolation trial, many fungal species was isolated and detected from bean seeds and the results were identical with Salgado *et al.* (1995) and Bruchera and Comacho (2000). *Alternaria alternata*, *Aspergillus parasiticus* and *Aspergillus flavus* were the most prominent of isolated fungi and this may be due to early infection in the field and/or during storage. These results were similar to a large extent with the findings of Silva *et al.* (2008). These pathogenic seed-borne fungi may be caused seed rot and seedling damping-off and excessive fungal growth of these fungi in the field can result in dark brown discoloration of the seed (Arafa *et al.*, 2002).

The Alternariol (AOH) toxin was produced by *A. alternate*. The highest concentration of this toxin was 85 μg kg^{-1} in discolored seeds of bean. The contamination with Alternariol associated with Aflatoxins produced by *A. flavus* or *A. parasiticus*. The results was parallel as results reported in cowpea by Zohri (1993), cowpea and garden pea (El-Kady *et al.*, 1996).

These levels were lower than those early detected by Stinson *et al.* (1980) when they testing mycotoxin production by *Alternaria* species on apples, tomatoes and beans. Such variations however, could be due to the differences in the host plant used. Values reported are the averages of three experiments.

The reduction in aflatoxin production as a result of double infection might due to that *A. alternata* would either degrade aflatoxin or compete with *A. parasiticus* for precursors of aflatoxin biosynthesis, since such precursors could be used to synthetize (AOH) toxin. *A. alternata* could also secrete some substance that specifically inhibits aflatoxin synthesis. These results were similar to that obtained by Dalcero *et al.* (1989) and Boller and Schroeder (1974). When studied microorganism interaction between *A. parasiticus* and *A. alternate* as well as *Aspergillus candidus*.

CONCLUSION

It has been concluded from this study that mycotoxins affect on the seed physiology and there is a direct relationship between the rate of mycotoxins (AF, AOH and ZON) contamination and the degree of discoloration. The presence of more than one fungal type together may reduce the content of mycotoxins.

REFERENCES

Abdulsalaam, S. and K.C. Shenge, 2011. Seed borne pathogens on farmer-saved sorghum (*Sorghum bicolor* L.) seeds. J. Stored Prod. Postharvest Res., 2: 24-28.

Alkahtani, M., M.A. El-Naggar, S.A. Omer, Eman, M. Abedl-Kareem and M.F. Ammar, 2011. Effect of Toxic *Fusarium moniliforme* on Some Biochemical Component of some Date Palm Cultivars Am. J. Food Technol., 6: 730-741.

Alwakeel, S.S. and L.A. Nasser, 2011. Microbial contamination and mycotoxins from nuts in Riyadh, Saudi Arabia. Am. J. Food Technol., 6: 613-630.

Arafa, M.K.M., M.A. Heweidy, K.M. Morsy and A.A. Ismail, 2002. Pathological and histopathological studies and toxin production of soybean seeds infected with *Alternaria alternate*. Egypt. J. Applied Sci., 17: 432-445.

Arafa, M.K.M., S.E. Zohri, S.E. Botros, M.G. Mosaad and I.A. Ismail, 1998. Germination, quality and production of toxin in discolored durum wheat seed infected with seed borne fungi. Egypt. J. Applied Sci., 13: 620-635.

Barnett, H.L. and B.B. Hunter, 1972. Illustrated Genera of Imperfect Fungi. 3rd Edn., Burgess Publishing Company, Minneapolis, MN., USA., Pages: 331.

Bechtel, D.B., L.A. Kalekau, R.L. Gaines and I.M. Seltz, 1989. The effects of *Fusarium graminearum* infection on wheat seeds. Cereal Chem., 62: 191-197.

Boller, R.A. and H.W. Schroeder, 1974. Influence of *Aspergillus canolidus* on production of aflatoxin in rice by *Aspergillus parasiticus*. Phytopathology, 64: 121-123.

Bottalico, A., A. Logarieco and A. Visconti, 1989. Fusarium species and their mycotoxin in infected corn in italy. Mycopathologie, 107: 85-92.

Brown, W., 1924. Two mycological methods method. II. A method of isolated single strain of fungi by cutting a hyphal tip. Ann. Bot., 38: 402-404.

Bruchera, R.A. and L. Comacho, 2000. Common bean reaction to *Fusarium oxyspoum* f. sp. Phaseoli, the cause of sever vascular wilt in central Africa. J. Phytaphol., 148: 39-45.

Bryden, W.L., 2007. Mycotoxins in the food chain: Human health implications. Asia Pac. J. Clin. Nutr., 16: 95-101.

Christensen, C.M., 1967. A note on invasion of durum wheat by storage fungi. Cereal Chem., 44: 100-102.

Dalcero, A., S. Chulze, M. Etcheverry, C. Farnochi and E. Varsavsky, 1989. Aflatoxins in sunflower seeds: Influence of *Alternaria alternate* on aflatoxin production by *Aspergilus parasiticus*. Mycopathlogia, 108: 31-35.

Dorner, J.W., 2008. Management and prevention of mycotoxins in peanuts. Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess., 25: 203-208.

El-Kady, I.A., S.S.M. El-Maraghy and A.A. Zohi, 1996. Aflatoxin formation and varietal difference of cow pea (*Vigna unguiculata* (L.) Walp.) and garden pea (*Pisum sativum* L.) cultivars. Mycopathologia, 133: 185-188.

El-Mougy, N.S., 2001. Field application of certain biological and chemical approaches on controlling bean wilt diseases. Egypt. J. Phytopathal., 29: 69-78.

Ezekiel, W.N., 1930. Modified procedure with the kiett single spore method. Phytopathol., 20: 583-586.

FAO, 2009. Statistic series. Food and Agricultural Organization of the United Nations, Rome.

Govindappa, M., V.R. Rai and S. Lokesh, 2011. *In vitro* and *in vivo* responses of different treating agents against wilt disease of safflower. J. Cereals Oilseeds, 2: 16-25.

Hemannavar, V., 2008. Studies on seed borne aspects of anthracnose of chilli and its management. M.Sc. Thesis, University of Agricultural Sciences, Dharwad.

ISTA, 1976. International rules for seed testing. Seed Sci. Technol., 4: 3-49.

Icishahayo, D., J. Sibiya, S. Dimbi, I.C. Madakadze, W. Manyangarirwa and B. Chipindu, 2009. Assessment of quality and health of field bean seeds home-saved by smallholder farmers. Afr. Crop Sci. Conf. Proc., 9: 609-615.

Mangala, U.N., K.R.N. Reddy, L. Singotamu, P.M.S. Chary, C.S. Reddy and K. Muralidharan, 2006. Aspergilli colonize and produce aflatoxin B1 in discolored rice grains. J. Mycol. Plant Pathol., 36: 418-426.

Melchert, H.U. and E. Pabel, 2004. Reliable identification and quantification of trichothecenes and other mycotoxins by electron impact and chemical ionization-gas chromatography-mass spectrometry, using an ion-trap system in the multiple mass spectrometry mode: Candidate reference method for complex matrices. J. Chromatogr. A, 1056: 195-199.

Patkowska, E., 2006. Effectiveness of grapefruit extract and *Pythium oligandrum* in the control of bean and peas pathogens. J. Plant Protect. Res., 46: 15-28.

Philippoussis, A.N., 2009. Biotechnology for Agro-Industrial Residues Utilisation: Utilisation of Agro-Residues. In: Biotechnology for Agro-Industrial Residues Utilisation, Nigam, P.S. and A. Pandey (Eds.). Springer, New York, ISBN: 9781402099410, pp: 163-196.

Pitt, J.I., 2000. Toxigenic fungi and mycotoxins. Br. Med. Bull., 56: 184-192.

Reddy, K.R.N., C.S. Reddy and K. Muralidharan, 2005. Characterization of aflatoxin B1 produced by *Aspergillus flavus* isolated from discolored rice grains. J. Mycol. Plant Pathol., 35: 470-474.

Reddy, K.R.N., S.B. Nurdijati and B. Salleh, 2010. An overview of plant-derived products on control of mycotoxigenic fungi and mycotoxins. Asian J. Plant Sci., 9: 126-133.

Salgado, M., D. Schwartz and M.A. Brick, 1995. Inheritance of resistance to a colorabo race of *Fusarium oxysporum* f. sp. phaseaoli and inoculation in dry bean under greenhouse conditions. Su. Phytopathologia, 2: 181-184.

Schothorst, R.C., A.A. Jekel, H.P. van Egmond, A. de Mul, P.E. Boon and J.D. van Klaveren, 2005. Determination of trichothecenes in duplicate diets of young children by capillary gas chromatography with mass spectrometric detection. Food Addit. Contam., 22: 48-55.

Silva, C.F., L.R. Batista and R.F. Schwan, 2008. Distribution of filamentous fungi during fermentation, drying and storage of coffee (*Coffea arabica* L.) beans. Braz. J. Microbiol., 39: 521-526.

Stinson, E.E., D.D. Billis, S.F. Osman, J. Sicilliano, M.J. Ceponis and E.G. Hesiler, 1980. Micotoxin production by alternaria species grown on apples, tomatos and blueberries. J. Agric. Food Chem., 28: 960-963.

Tanaka, T., A. Yoneda, S. Inoue, Y. Sugiura and Y. Ueno, 2000. Simultaneous determination of trichothecene mycotoxins and zearalenone in cereals by gas chromatography-mass spectrometry. J. Chromatogr. A., 882: 23-28.

Youssef, M.S., 2009. Natural occurrence of mycotoxins and mycotoxigenic fungi on libyan corn with special reference to mycotoxin control. Res. J. Toxins, 1: 8-22.

Youssef, M.S., E.M. El-Mahmoudy and M.A.S. Abubakr, 2008. Mesophilic Fungi and mycotoxins contamination of libyanm cultivated four fabaceae seeds. Res. J. Microbiol., 3: 520-534.

Zhang, C.C., 1992. The pathogen and control of kidney ben wilt disease. Acta. Phytophylactica Sin., 19: 107-110.

Zohri, A.A., 1993. Studies on some cowpea cultivars II. Suitability for *Aspergillus flavus* growth and aflatoxin production. Qatar Univ. Sci. J., 13: 57-62.